

ADVANCED FUNCTIONAL MATERIALS

Supporting Information

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A Chemically Defined Hydrogel for Human Liver Organoid Culture

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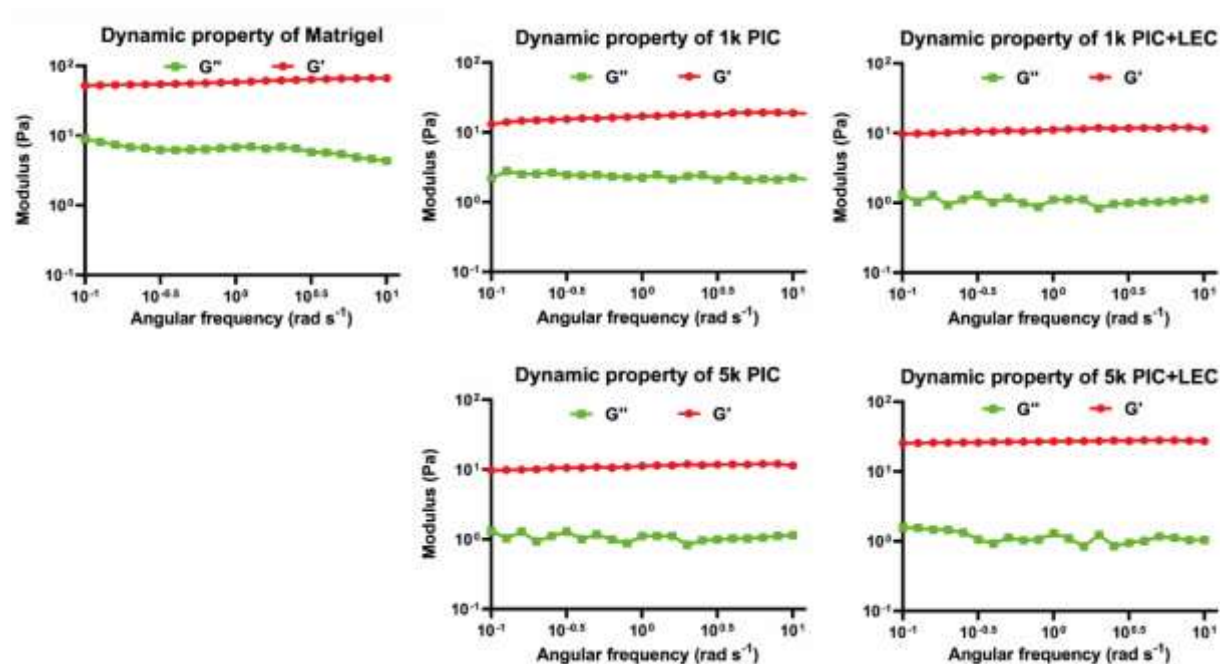


Figure S1. Frequency dependent modulus of Matrigel and PIC hydrogels.

A frequency sweep mode was applied to characterize the dynamic property of Matrigel, 1k PIC, 1k PIC+LEC, 5k PIC and 5k PIC+LEC with fixed strain (1%). Storage modulus G' and loss modulus G'' of hydrogels were recorded in frequency sweep mode after 10 min of gelation at 37°C. For all hydrogels, G' was consistently higher than G'' in all frequency ranges (10 to 0.1 rad s^{-1}), indicating that tested hydrogels were stable.

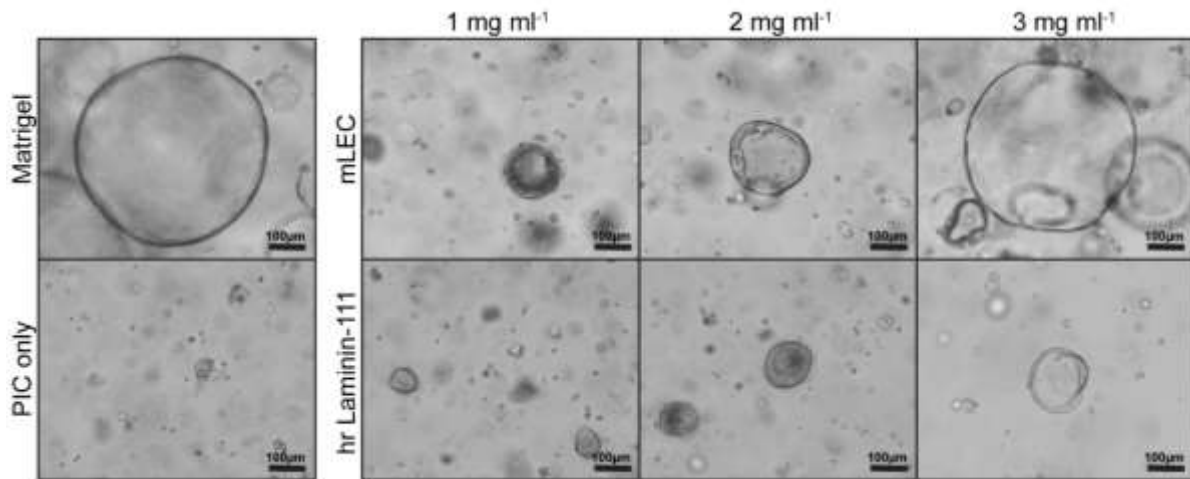


Figure S2. Human recombinant laminin-111 can substitute LEC.

Organoids from three different donors were cultured for 14 days in Matrigel, PIC-plain or 1k PIC supplemented with either LEC or human recombinant Laminin (hrLaminin-111). Light microscopy pictures taken at day 11 of culture are of the donor for which hrLaminin-111 had the least efficient proliferative effect compared to LEC, as a comparison to the donor shown in Figure 6a (N=2).

Table S1. List of 100 most differentially-expressed genes in DM compared to EM.

ENSG00000165376	CLDN2	claudin 2
ENSG00000167653	PSCA	prostate stem cell antigen
ENSG00000131747	TOP2A	DNA topoisomerase II alpha
ENSG00000059804	SLC2A3	solute carrier family 2 member 3
ENSG00000163209	SPRR3	small proline rich protein 3
ENSG00000087916	SLC6A14	solute carrier family 6 (amino acid transporter), member 14
ENSG00000175538	KCNE3	potassium voltage-gated channel subfamily E regulatory subunit 3
ENSG00000130600	H19	H19 imprinted maternally expressed transcript
ENSG00000205420	KRT6A	keratin 6A
ENSG00000114455	HHLA2	HERV-H LTR-associating 2
ENSG00000148053	NTRK2	neurotrophic receptor tyrosine kinase 2
ENSG00000188042	ARL4C	ADP ribosylation factor like GTPase 4C
ENSG00000233041	PHGR1	proline, histidine and glycine rich 1
ENSG00000078401	EDN1	endothelin 1
ENSG00000038427	VCAN	versican
ENSG00000163817	SLC6A20	solute carrier family 6 member 20
ENSG00000169469	SPRR1B	small proline rich protein 1B

ENSG00000165272	AQP3	aquaporin 3 (Gill blood group)
ENSG00000188488	SERPINA5	serpin family A member 5
ENSG00000115129	TP53I3	tumor protein p53 inducible protein 3
ENSG00000131746	TNS4	tensin 4
ENSG00000152990	ADGRA3	adhesion G protein-coupled receptor A3
ENSG00000197506	SLC28A3	solute carrier family 28 member 3
ENSG00000167755	KLK6	kallikrein related peptidase 6
ENSG00000213759	UGT2B11	UDP glucuronosyltransferase family 2 member B11
ENSG00000159167	STC1	stanniocalcin 1
ENSG00000120885	CLU	clusterin
ENSG00000188549	CCDC9B	coiled-coil domain containing 9B
ENSG00000007062	PROM1	prominin 1
ENSG00000170271	FAXDC2	fatty acid hydroxylase domain containing 2
ENSG00000198074	AKR1B10	aldo-keto reductase family 1 member B10
ENSG00000131016	AKAP12	A-kinase anchoring protein 12
ENSG00000156535	CD109	CD109 molecule
ENSG00000105290	APLP1	amyloid beta precursor like protein 1
ENSG00000125398	SOX9	SRY-box transcription factor 9
ENSG00000137857	DUOX1	dual oxidase 1
ENSG00000160181	TFF2	trefoil factor 2
ENSG00000169035	KLK7	kallikrein related peptidase 7
ENSG00000019582	CD74	CD74 molecule
ENSG00000196352	CD55	CD55 molecule (Cromer blood group)
ENSG00000171617	ENC1	ectodermal-neural cortex 1
ENSG00000137673	MMP7	matrix metalloproteinase 7
ENSG00000169876	MUC17	mucin 17, cell surface associated
ENSG00000108375	RNF43	ring finger protein 43
ENSG00000167244	IGF2	insulin like growth factor 2
ENSG00000104529	EEF1D	eukaryotic translation elongation factor 1 delta
ENSG00000074370	ATP2A3	ATPase sarcoplasmic/endoplasmic reticulum Ca ²⁺ transporting 3
ENSG00000101842	VSIG1	V-set and immunoglobulin domain containing 1
ENSG00000120437	ACAT2	acetyl-CoA acetyltransferase 2
ENSG00000074276	CDHR2	cadherin related family member 2
ENSG00000203697	CAPN8	calpain 8
ENSG00000196611	MMP1	matrix metalloproteinase 1
ENSG00000140332	TLE3	TLE family member 3, transcriptional corepressor
ENSG00000088992	TESC	tescalcin
ENSG00000180861	LINC01559	long intergenic non-protein coding RNA 1559
ENSG00000158201	ABHD3	abhydrolase domain containing 3
ENSG00000140274	DUOXA2	dual oxidase maturation factor 2
ENSG00000134419	RPS15A	ribosomal protein S15a
ENSG00000227195	MIR663AHG	MIR663A host gene
ENSG00000215182	MUC5AC	mucin 5AC, oligomeric mucus/gel-forming
ENSG00000101444	AHCY	adenosylhomocysteinase
ENSG00000134324	LPIN1	lipin 1
ENSG00000109084	TMEM97	transmembrane protein 97
ENSG00000105388	CEACAM5	carcinoembryonic antigen related cell adhesion molecule 5

ENSG00000196924	FLNA	filamin A
ENSG00000117707	PROX1	prospero homeobox 1
ENSG00000140279	DUOX2	dual oxidase 2
ENSG00000165672	PRDX3	peroxiredoxin 3
ENSG00000163428	LRRC58	leucine rich repeat containing 58
ENSG00000167508	MVD	mevalonate diphosphate decarboxylase
ENSG00000204616	TRIM31	tripartite motif containing 31
ENSG00000080503	SMARCA2	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 2
ENSG00000100342	APOL1	apolipoprotein L1
ENSG00000073737	DHRS9	dehydrogenase/reductase 9
ENSG00000205413	SAMD9	sterile alpha motif domain containing 9
ENSG00000266714	MYO15B	myosin XVb
ENSG00000169213	RAB3B	RAB3B, member RAS oncogene family
ENSG00000112902	SEMA5A	semaphorin 5A
ENSG00000065618	COL17A1	collagen type XVII alpha 1 chain
ENSG00000099139	PCSK5	proprotein convertase subtilisin/kexin type 5
ENSG00000147883	CDKN2B	cyclin dependent kinase inhibitor 2B
ENSG00000169174	PCSK9	proprotein convertase subtilisin/kexin type 9
ENSG00000104365	IKBKB	inhibitor of nuclear factor kappa B kinase subunit beta
ENSG00000079112	CDH17	cadherin 17
ENSG00000015520	NPC1L1	NPC1 like intracellular cholesterol transporter 1
ENSG00000164120	HPGD	15-hydroxyprostaglandin dehydrogenase
ENSG00000175793	SFN	stratifin
ENSG00000042980	ADAM28	ADAM metalloproteinase domain 28
ENSG00000198431	TXNRD1	thioredoxin reductase 1
ENSG00000067082	KLF6	Kruppel like factor 6
ENSG00000131069	ACSS2	acyl-CoA synthetase short chain family member 2
ENSG00000109929	SC5D	sterol-C5-desaturase
ENSG00000179674	ARL14	ADP ribosylation factor like GTPase 14
ENSG00000160752	FDPS	farnesyl diphosphate synthase
ENSG00000160285	LSS	lanosterol synthase
ENSG00000112715	VEGFA	vascular endothelial growth factor A
ENSG00000165105	RASEF	RAS and EF-hand domain containing
ENSG00000067064	IDI1	isopentenyl-diphosphate delta isomerase 1
ENSG00000010438	PRSS3	serine protease 3
ENSG00000128039	SRD5A3	steroid 5 alpha-reductase 3

Table S2. GO terms of biological processes related to liver functions.

ID	Name	Genes from Input (97 genes)	Genes in Annotation	p-value
GO:0008202	steroid metabolic process	15	366	2.34E-10
GO:1901615	organic hydroxy compound metabolic process	18	586	3.41E-10
GO:0016126	sterol biosynthetic process	8	83	6.31E-09
GO:0006694	steroid biosynthetic process	11	213	6.40E-09
GO:0008299	isoprenoid biosynthetic process	6	31	7.12E-09
GO:0008610	lipid biosynthetic process	18	748	1.57E-08
GO:0016125	sterol metabolic process	10	181	1.71E-08
GO:0006066	alcohol metabolic process	13	397	5.80E-08
GO:0006629	lipid metabolic process	24	1471	8.43E-08
GO:0006695	cholesterol biosynthetic process	7	77	8.80E-08
GO:1902653	secondary alcohol biosynthetic process	7	78	9.63E-08
GO:0008203	cholesterol metabolic process	9	165	1.03E-07
GO:1902652	secondary alcohol metabolic process	9	184	2.60E-07
GO:0042445	hormone metabolic process	10	264	5.82E-07
GO:0044283	small molecule biosynthetic process	16	763	6.93E-07
GO:0042127	regulation of cell population proliferation	26	1921	8.41E-07
GO:0008284	positive regulation of cell population proliferation	19	1096	9.92E-07
GO:1901617	organic hydroxy compound biosynthetic process	10	281	1.03E-06
GO:0050810	regulation of steroid biosynthetic process	7	111	1.09E-06
GO:0046165	alcohol biosynthetic process	8	180	2.55E-06
GO:0016999	antibiotic metabolic process	7	131	3.31E-06
GO:0017000	antibiotic biosynthetic process	4	23	4.17E-06
GO:0030855	epithelial cell differentiation	16	880	4.40E-06
GO:0045540	regulation of cholesterol biosynthetic process	5	52	5.07E-06
GO:0106118	regulation of sterol biosynthetic process	5	52	5.07E-06
GO:0006720	isoprenoid metabolic process	7	143	5.91E-06
GO:0019218	regulation of steroid metabolic process	7	145	6.47E-06
GO:0017001	antibiotic catabolic process	5	59	9.50E-06
GO:2000147	positive regulation of cell motility	13	644	1.28E-05
GO:0042743	hydrogen peroxide metabolic process	5	63	1.31E-05
GO:0046890	regulation of lipid biosynthetic process	8	225	1.32E-05

Table S3. List of primers used.

Target	Forward primer	Reverse primer	Annealing Temp [°C]	Product size [bp]
<i>HPRT</i>	ATAAGCCAGACTTTGTTGGA	CTCAACTTGA ACTCTCATCTTAGG	57	192
<i>RPL19</i>	ATGAGTATGCTCAGGCTTCAG	GATCAGCCCATCTTTGATGAG	60	150
<i>ALB</i>	GTTCGTTACACCAAGAAAGTACC	GACCACGGATAGATAGTCTTCTG	64	144
<i>CYP3A4</i>	TTTTGTCCTACCATAAGGGCTTT	CACAGGCTGTTGACCATCAT	64	92
<i>MRP2</i>	GGGATCTCTTCCACACTGGAT	CATACAGGCCCTGAAGAGGA	64	91
<i>LGR5</i>	GCAGTGTTACCTTCCC	GGTCCACACTCCAATTCTG	64	266

Table S4. List of antibodies used.

Primary antibodies diluted in antibody diluent (Dako) for IF					
Antigen	Source and cat. number	Raised in	Dilution	Antigen retrieval	Incubation
Ki67	Thermo Scientific, RM-9106-S	rabbit	1:75	TE buffer for 30 min at 98°C	O/N at 4°C
E-cadherin	BD Bioscience, 610181	mouse	1:100		
PCNA	Santa Cruz, sc-56	mouse	1:300		
CK19	Abcam, ab76539	rabbit	1:150		
Secondary antibodies diluted in antibody diluent (Dako)					
Antigen	Source and cat. number	Raised in	Dilution	Incubation	
Anti-mouse Alexa 488	ThermoFisher A-11029	goat	1:200	1 h at RT	
Anti-rabbit Alexa 488	ThermoFisher A-11034				
Anti-mouse Alexa 568	ThermoFisher A-11004				
Anti-rabbit Alexa 568	ThermoFisher A-11036				